Method for Scanner Control in at least one Scan Axis in a Laser Scanning Microscope

Methods for scanner control in a laser scanning microscope are described, for example, in DE 19702752 C2.

Despite calibration (for example, by a linear electrical signal) of the scanner, fraying of vertical lines by phase differences between the forward and back scan (bidirectional deviations bidir) occurs, caused by:

1. Long-term changes of scanner response
2. Temperature/load-related fluctuations
3. Zoom dependence of scanner response.

This is to be improved by the invention.

This task is solved by the features of Claim 1.

Preferred modifications are the object of dependent claims.

The underlying idea of the invention is to carry out evaluation of a scan image by investigating it for bidirectional deviations and conducting a continuous correction of the coefficients for scanner control during microscope operation (for example, between scans).

The invention is further explained below by means of schematic representations.

Figure 1 shows a laser scanning microscope LSM with a scan module SC and a detection module DE.

Additional details are known from DE 19702753 A1.

Control of the SC and DE, as well as feedback of detection of the control state occur via a control unit AS, for example, a real-time computer.

AS is connected to a PC as user interface.

Image evaluation according to the invention and a change in control data for the scanner can occur in the real-time computer or PC.

Figure 2 shows an example of the basic principle.

Here the partial images B1, B2 for the forward and back scan are shown by means of a scanned cross grid sample with grid strips GS, in Figure 2a in the X scanner direction and in Figure 2b in the Y scanner direction. Partial images B1, B2 are brought together and correlated with each other, i.e., made congruent by displacement and the correction values determined.

Figure 3 shows that the cut direction of the strip-like partial images Bs to determine the error of the X scanner occurs perpendicular to the X direction, i.e. in the Y direction (unrotated scan).

Since the Y scanner is at rest between scan lines, determination of the error in the Y direction is not possible here.

A detailed evaluation of image distortions caused by line movement of the scanner is possible if the image is cut into strips perpendicular to the scan lines and each strip evaluated by itself. The overall trend of the bidirectional deviations within a scan line is then obtained. Knowledge of this trend permit better correction than a simple displacement between forward and back lines constant over the entire image.

Unrotated scan variant

This means that one of the scanners operates as a fast line scanner (hereafter referred to as X scanner) and the other scanner as a slow line scanner (hereafter referred to as Y scanner). The Y scanner advantageously jumps during the reversal phases of the X scanner from line to line (line advance) and is at rest during actual data recording. This rotational direction of the scan is referred to subsequently as 0° scan angle. The cut direction for the strips is parallel to the image edge and also parallel to the Y direction for 0° scan angle.

The displacement direction to determine the error of the X scanner is parallel to the scan direction of the X scanner, i.e., the two partial images of the even and odd scan lines (forward and back scan) are displaced relative to each other along the direction in the figure that is stipulated by movement of the X scanner, i.e., parallel to the scan lines at 0° scan.

A determination of the error of the Y scanner is not possible at 0° scan (Y scanner at rest during scan lines).

Figure 4 shows the case of a rotated scan (scan angle not equal to 0°, 180°, 90° and -90°). In this case both scanners operate synchronously as line scanner. Between the scan lines both scanners advantageously jump during the reversal times on both scanners to the next line. By adjusting the scan amplitudes of both scanners, the scan angle is adjusted (orientation of the scan line in the sample). In this case the cut direction for the strip-like partial images occurs at an angle to the scan directions X and Y. The cut direction also lies here essentially in the edge direction of the scanned image.

To determine the error of the X scanner a shift of the partial images from the forward and back scan in the X direction occurs (direction of the X scanner, not direction of the scan lines), to determine the Y error in the Y direction (direction of the X scanner, not direction of the scan lines).

The corresponding correction is determined in each case and the correction vector determined from both values.

Rotated scan variant, i.e., scan angle not equal to 0°, 90°, -90° and 180°:

The X scanner and Y scanner move quickly and both scanners jump a little bit between lines.

When both scanners start simultaneously with the same amplitude, the light point in the sample does not run from left to right but from bottom left to top right (for example) → 45° rotated scan. If the Y scanner runs with only half amplitude, the light spot goes from bottom left to the half top right → about 22° scan angle … between the rotated scan lines, both scanners jump a little bit (constant offset of movement superimposed) so that the next scan line lies next to the last one.

The cut direction for the strips is also advantageously (?) parallel to the image edge, perpendicular to the direction of the scan lines.

The displacement direction to determine the error of the X scanner is parallel to the scan direction of the X scanner, i.e., the two partial images of the even and odd scan lines (forward and back scan) are displaced relative to each other along a direction of the image stipulated by movement of the X scanner (the direction along which the light spot would move in the sample if the X scanner were moved alone).

The displacement direction to determine the error of the Y scanner is parallel to the scan direction of the Y scanner.

It can also be useful, in order to determine the time trend of the image distortion, to also break down the image in parallel to the direction of the scan lines.

In a rotated scan the scanner is scanned not along the scan lines but "obliquely" through the image. The directions of both scan movements are then essential for the bidirectional deviations, not the direction of the scan lines.

Determination of the bidirectional deviations occurs as follows:

- Cutting of the image into (for example) 10 equally wide strips, preferably along the slow scan axis (cut perpendicular to the fast axis)
- Cutting of the strips in the two partial images from the forward scan and back scan (if necessary filling in the missing lines with the average value of the adjacent lines)
- Calculation of (one-dimensional) cross-correlation functions of the two partial images along the two scan directions (the following evaluation occurs for both scan directions in order to be able to correct both scanners)
    o during displacement of the two partial strips relative to each other, all of the image data can actually be utilized, i.e., missing data in the edge region of the partial images are taken from the neighboring strips
- Evaluation of these correlation functions:

- o position of the maximum: at this displacement length the two partial images best fit each other, i.e., this displacement is the desired correction of the bidirectional deviation on the image position on which the strip is situated
- o half-width and half-height of the peak: gauge of the accuracy of measurement. The cross correlation function is normalized, for example, to the autocorrelation(s) of the two partial images, i.e., correlation 1 means that the two partial images agree exactly during this displacement.
- At rotation angles that deviate less than (for example) 10° from the main axis of the scanner, only this scanner should be evaluated (for example, in a 0° scan only the X scanner can be evaluated)
- The position of the peaks of the individual image strips yield a curve per scan direction (x, y) with, for example, 10 support sites (number of image strips) for the bidirectional deviation in the image.
- Depending on the accuracy of the measurement (half-width and half-height of the cross-correlation peaks) these support sites should advantageously be marked with weighting factors or, if unusable, discarded.

Correction of the scanner control occurs advantageously via the coefficients of a Fourier series from the signal curves of the scanner control signals.

The correction of the scanner coefficient then occurs as follows:

- Determination of the scanner coefficient that best corrects the found deviation curve Correlation of the measured deviation curve with $\sin(1f)$, $\sin(2f)$, $\sin(3f)$, ... (which coefficient yields the maximum correlation with the measured deviation curve?)
- The phase error of the coefficient can be calculated from the correlation value (large amplitude of the deviation curve = large phase error of the coefficient) When the deviation is caused only by a coefficient, a sine curve with nodal points at the reversal points of the scanner is always present (outside of the image)

- Correction of the phase of the coefficient (amplitude is advantageously not changed in order to obtain the long-term linearity of scanner movement)
- The phase of the concerned coefficient could also not be fully corrected, depending on the quality of the measurement (weighting factors, see above)
  The finally valid correction then occurs, for example, during the next scan images.
- In this way with each scan (at least) one coefficient could be corrected. By correlation of the deviation curve with several scanner frequencies, several coefficients can also be corrected in one step (depending on the accuracy of measured values).

Over time a parameter field can be produced on the control computer of the microscope with scanner coefficients that are permanently adjusted.

The parameters are, for example: speed, zoom (for example zoom 0.7, 0.8, 1, 2, 4, 8).

If an intermediate zoom is used, interpolation can occur from neighboring coefficients and in similar fashion the neighboring coefficients (also with weighting) can be adjusted after a deviation measurement.

The parameter field on the hard disk can only slowly change advantageously (for example, weighted average between daily average and present value in the files).

In addition, a further relatively rapidly variable parameter field can occur in that a reaction occurs to temperature fluctuations during the day (according to possibility, evaluated after each image).

A correction of the active coefficient could also occur during deviations greater than a stipulated threshold (for example, a value adjustable by the user).

This parameter field is also recorded and stored.

Depending on the deviation width and quality of this day-parameter on completion of the LSM program, the field stored on the hard disk could be adjusted (possible parameters for weighting: deviation width of the measured deviations, value number, operating hours, etc.). The on-line correction can be switched off, i.e., the user can have the possibility of activating or suppressing the mechanism of automatic correction of the scanner control. The original calibration set from the plant can be stored for evaluations of development of the mechanical and electrical behavior of the scanner and the copy can be permanently stored. The parameter set on the hard disk can also be reset (by means of the original calibration).

A comparison of the formed parameter set with the original set could lead, for example, to a recommendation on the screen for the user "Please calibrate the scanner again" (the threshold value could be different according to scan speed).

In multichannel images (during the scan different data are recorded, for example, for each image pixel with several detectors) all channels could be evaluated and compared separately (the scanner-related image distortion should be the same in all images).

Through the described correction, in addition to approved bidirectionality, an improvement in linearity is also possible, since the coefficients can be exactly shifted with reference to their phase.